ORIGINAL ARTICLE

Increased urinary excretion of 8-hydroxydeoxyguanosine in engine room personnel exposed to polycyclic aromatic hydrocarbons

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Background: Previous investigations indicate that engine room personnel on ships are exposed to polycyclic aromatic hydrocarbons (PAH) from oil and oil products, with dermal uptake as the major route of exposure. Several PAH are known carcinogens and mutagens.
Aims: To investigate the urinary excretion of a marker for oxidative DNA damage, 8-hydroxydeoxyguanosine (8OHdG), in engine room personnel, and to study the association between 8OHdG and 1-hydroxypyrene (1OHP), a biological marker for PAH exposure.
Methods: Urine samples were collected from engine room personnel (n = 36) on 10 Swedish and Norwegian ships and from unexposed controls (n = 34) with similar age and smoking habits. The exposure to oils, engine exhaust, and tobacco smoke 24 hours prior to sampling was estimated from questionnaires. The urinary samples were frozen for later analyses of 8OHdG and 1OHP by high performance liquid chromatography.
Results: Excretion in urine of 8OHdG (adjusted to density 1.022) was similar for controls (mean 18.0 nmol/l, n = 33), and for those who had been in the engine room without skin contact with oils (mean 18.7 nmol/l, n = 15). Engine room personnel who reported skin contact with oil had increased excretion of 8OHdG (mean 23.2 nmol/l, n = 19). The difference between this group and the unexposed controls was significant. The urinary levels of ln 1OHP and ln 8OHdG were significantly correlated, and the association was still highly significant when the effects of smoking and age were accounted for in a multiple regression analysis.
Conclusion: Results indicate that exposure to PAH or possibly other compounds from skin contact with oils in engine rooms may cause oxidative DNA damage.

Several studies have shown that engine room personnel on ships have an increased mortality from cancer of the lung and urinary bladder.[1–6] The increased risks could not entirely be explained by tobacco smoking. Exposure to asbestos is probably the main cause of the increased risk of lung cancer, but polycyclic aromatic hydrocarbons (PAH) and nitroarenes could also be causal factors. Polyaromatic hydrocarbons constitute a large group of chemical substances found in, for example, diesel exhaust, soot, and some heavy fuel oils. Lubricating oils in engines are often contaminated with PAH from combustion products.

Several polycyclic aromatic hydrocarbons can cause cancer, especially of the lung, skin, and possibly urinary bladder.[7] Exposure can occur by inhalation but also through dermal uptake.[8] The pyrene metabolite 1OHP in urine can be used as a biological marker for exposure to PAH.[9–12]

The metabolism of PAH, for example, benzo[a]pyrene, is complex and DNA damage may occur from, for example, quinone metabolites, both by covalent binding of the metabolite to DNA and by generation of reactive oxygen species from one electron redox cycling which may cause oxidative damage to DNA.[13–15]

8-Hydroxydeoxyguanosine (8OHdG) in urine is a biological marker of oxidative stress on DNA.[16–18] There is evidence that oxidative stress can be involved in carcinogenesis.[17 19]

We have previously reported an increased excretion of 1OHP in urine among engine room personnel on 10 Swedish and Norwegian ships where the main exposure route seems to be through dermal uptake from lubricant and heavy fuel oils on skin.[20] In that study duplicate samples of urine from exposed subjects and unexposed controls were frozen and stored. We subsequently analysed the samples for 8OHdG in order to study whether exposure to PAH or other compounds in the oils were associated with genotoxic effects from free radical oxygen species. The results of the extended analyses are reported in this paper.

METHODS

Urinary samples were collected from seamen on five Swedish and five Norwegian ships in this cross sectional study. Five were passenger ships, two were roll on-roll off ships, two product tankers, and one a container ship. The ships were built between 1956 and 1993 and the sizes range from 5000 to 50 000 dead weight tonnes. Four of the ships were of an older type with an engine room without a separate control room. All the engine room personnel on the ships were invited to participate (n = 51). Seamen employed on the same ships with similar age and smoking habits as the exposed group were selected as controls (n = 47).

The exposure classification was based on self reported exposure to oils on the skin and probable inhalation of oil mist and engine exhaust during work in the engine rooms. Questionnaires were used to obtain data on age, occupation, and exposure to possible sources of PAH the past 24 hours, use of personal protection equipment, and smoking habits. No medical examinations were performed in this study. The

Abbreviations: DNA, deoxyribonucleic acid; ln, natural logarithm (base = e); 8OHdG, 8-hydroxydeoxyguanosine; 1OHP, 1-hydroxypyrene; PAH, polycyclic aromatic hydrocarbons; SE, standard error

Main messages

- Exposure to PAH at comparatively low levels may cause oxidative DNA damage.
- The association between the urinary concentrations of 1-hydroxypyrene and 8-hydroxydeoxyguanosine indicate that the genotoxic effect is partly due to PAH or factors associated with PAH exposure.
- It is possible that exposure to PAH from skin contact with oils may contribute to the increased incidence of cancer reported among engine room personnel.

Policy implications

- Skin contact with oils containing PAH is a possible risk factor for cancer that should be considered.
- The uptake of PAH from oils could probably be reduced by using appropriate protection and by reducing the PAH content of the oils.

exposure of the crew to PAH was categorised according to their answers on the questionnaire. The categories were unexposed controls, meaning no known exposure to PAH in the past 24 hours; exposed degree I (engine room personnel—no oil on skin), meaning that they had been working inside the engine room the past 24 hours but had not experienced any contamination of the skin with oil during this period; and exposed degree II (engine room personnel—oil on skin), meaning that they had been working in the engine room during the past 24 hours and had experienced contamination of the skin with oil during this period.

We asked about exposure during the 24 hours before the urine sample was taken since most studies have reported an individual half life of 1OHP in the range 4–35 hours.[11 12 21] The kinetics of 8OHdG are not well known, but a study in workers exposed to benzene and oils indicated that there is a peak excretion within 24 hours after exposure.[22]

Established and standardised methods were used for the sampling and analyses of 1OHP and 8OHdG. A urine specimen of 50 ml was obtained from each subject. Aliquots of the samples (2×10 ml) were stored frozen at −20°C until laboratory analysis of the urine. Each sample was coded and analysed without knowledge of exposure status. The urine samples were analysed for 1OHP by the method described by Jongeneelen and colleagues.[20 23] The coefficient of variation for the analyses of 1OHP is 15% for a 25 nM standard, day to day. Urinary 8OHdG in urine samples was analysed by coupled column high performance liquid chromatography with electrochemical detection as described previously.[24] Previous studies have shown that the coefficient of variation for 8OHdG is 5–7% for duplicate samples and 8–23% between series.[25] 8OHdG is reported to be stable in urine stored at −20°C for at least one year.[26]

The urinary concentrations of 1OHP and 8OHdG were adjusted to a standardised density of 1.022, in order to compensate for differences in urinary flow rate. Most previous studies have used urinary concentration of creatinine to normalise for dilution, but it is known that the urinary excretion of creatinine shows considerable inter- and intra-individual variability and is influenced by, for example, urinary flow, protein intake, muscle mass, and severe muscular activity; serious doubts have been expressed as to the validity of creatinine for normalisation purposes.[27–29] If normalisation with respect to body mass, for example, is desired, it offers some advantages, but if the exposure dose is most important, it has serious drawbacks. The engine room personnel in our study had significantly higher concentrations of creatinine in their urine (mean 15.5 mmol/l) than the controls (mean 12.1 mmol/l). This could lead to false correlations in the analyses even if the excreted amounts of the biomarkers are the same among the exposed and controls. Urine density was, however, similar (1.023 and 1.021 respectively) and we chose this method to adjust for dilution. Density adjustments have been used in several recent publications.[25 30] Adjustment for density is considered only to be reliable in the range 1.010–1.035.[28] We excluded urinary samples with density values outside this range from the analyses.

Data were analysed with the SPSS statistical software package. The data were log transformed prior to the *t* tests since they had an approximate log normal distribution (according to P-P plots). Multiple regression analyses were also performed on log transformed data.

RESULTS

The exposed group consisted originally of 51 men employed in the engine rooms and the control group of 47 men employed in other positions on the same ships. We could not obtain enough urine to analyse both 1OHP (1OHP) and 8OHdG (8OHdG) from 14 of the exposed subjects and from 12 controls. One urine sample from the exposed group was not analysed for 8OHdG due to technical failure. Two urine samples in the exposed group and one in the control group were excluded due to low density (<1.010). Thus, 34 exposed men and 33 controls were included in the study. Nineteen of the exposed subjects reported skin contact with oils in the 24 hours prior to taking the urinary sample.

The mean age was somewhat higher in the control group than in the exposed groups, and engine room personnel with no skin contact with oils had a higher percentage of smokers than the other groups (table 1).

Protective gloves or facemasks were not used. A previous exposure study of PAH in the engine rooms showed no detectable levels of the 16 analysed PAH in 19 air samples; dermal uptake of oils was considered to be the major route of exposure.[20]

The mean urinary concentrations of 1OHP in non-smokers were low among unexposed controls and engine room personnel with no oil contamination of the skin during 24 hours prior to taking the urinary sample (table 2). Smokers had generally higher mean urinary concentrations of 1OHP than non-smokers, except in the group reporting oil contamination of their skin. The number of subjects in this group was, however, low (n = 6) as well as the non-smoking engine room personnel reporting no oil on skin, resulting in rather wide confidence intervals. Subjects who reported oil contamination of the skin the 24 hours prior to collecting the urinary sample had a higher mean concentration of 1OHP in their urine than the unexposed controls ($p < 0.001$, *t* test of log transformed data).

Multiple linear regression analysis showed a clear association between ln 1OHP and exposure group (unstandardised regression coefficient 0.62, SE 0.15; standardised regression coefficient 0.45; $p < 0.0004$) and smoking category (unstandardised regression coefficient 0.18, SE 0.08; standardised regression coefficient 0.24; $p = 0.03$, adjusted R^2 0.25).

The excretion of 8OHdG was higher among engine room personnel exposed to oils on their skin for the last 24 hours compared to the unexposed controls ($p = 0.03$, two sided *t* test of log transformed data) (table 2).

There was a statistically significant correlation (Pearson) between ln1OHP and ln 8OHdG ($r = 0.34$, $p = 0.005$) (fig 1).

Table 1 Age and smoking habits in different exposure groups

	n	Age*	Non-smokers	Cigarettes/day 1–10	11–10	>20
Unexposed controls	33	43 (23–59)	22 (67%)	3 (9%)	5 (15%)	3 (9%)
Engine room personnel						
No oil on skin	15	36 (17–52)	6 (40%)	2 (13%)	5 (34%)	2 (13%)
Oil on skin	19	37 (21–53)	13 (68%)	1 (5%)	3 (16%)	2 (11%)

*Mean (range).

Exclusion of the two outliers with high 1OHP and low 8OHdG increased the correlation only marginally ($r = 0.40$, $p = 0.001$).

The effect of ln 1OHP on ln 8OHdG when controlling for age was highly significant (unstandardised regression coefficient 0.12, SE 0.04; standardised regression coefficient 0.33; $p = 0.006$, adjusted R^2 0.11). When including other exposure variables and smoking in the model, the effect of ln 1OHP was still significant (table 3). Analyses for interaction between smoking and exposure showed non-significant effects and the interaction term was excluded from the final analysis. Exclusion of the two outliers increased the coefficient for ln 1OHP somewhat (unstandardised regression coefficient 0.15, SE 0.053; standardised regression coefficient 0.38; $p = 0.007$).

DISCUSSION

The urinary excretion of 8OHdG was highest among engine room personnel whose skin had been contaminated with oil, and the urinary levels of 8OHdG and 1OHP were significantly correlated. This indicates that exposure to PAH or factors associated with PAH exposure may contribute to an increased oxidative stress among engine room personnel on ships.

8OHdG is not a specific marker for PAH exposure but a general biomarker for oxidative stress on DNA and the nucleotide pool. Its formation and elimination is complex and dependent on many different factors such as oxygen uptake, exposure to radiation and chemical substances, enzyme polymorphism, and scavenger and DNA repair activity, leading to substantial intra- and inter-individual variations in its excretion.[31 32] This will generally lead to difficulties for detection of specific effects, but may also lead to spurious associations if there are systematic differences between the exposed and control groups.

The associations found in this study do not necessarily have to be causal. It is possible that agents or factors other than PAH have contributed to the oxidative stress and excretion of 8OHdG in urine, and the observed association may have been confounded by, for example, differences in workload or concomitant exposure to other genotoxic compounds, such as nitroarenes, which are formed during combustion and may have contaminated the lubricant oil. Nitroarenes have been reported to increase the formation of 8OHdG.[33] The results of the analyses indicate, however, that PAH or factors associated with PAH exposure may be of importance.

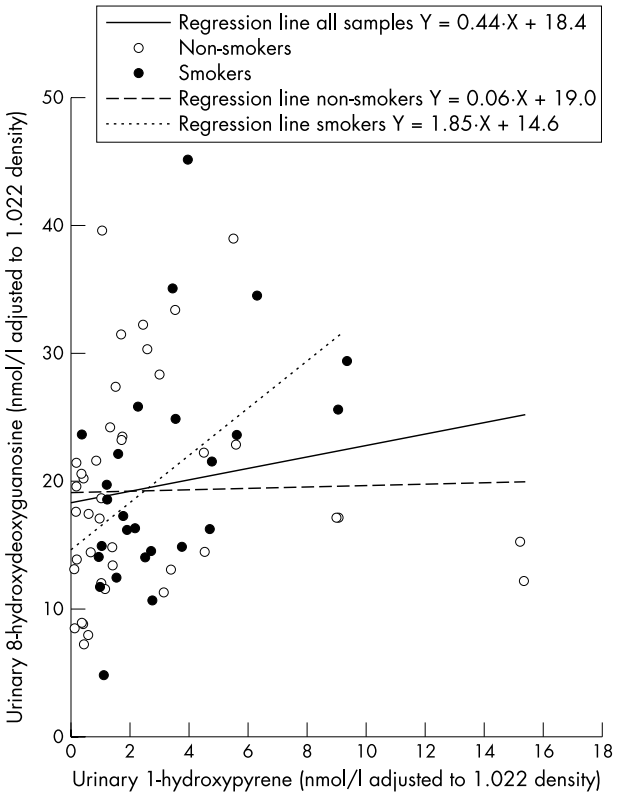

Figure 1 Scatterplot of urinary 8OHdG *v* urinary 1OHP (nmol/l, adjusted to 1.022 density).

The reported effects of physical exercise and body mass index on 8OHdG in urine often seem contradictory. Mild

Table 2 Urinary concentrations (nmol/l, adjusted to density 1.022) of 1OHP and 8OHdG in different exposure groups

	n	1OHP (95% CI)	8OHdG (95%CI)
Unexposed controls	33	1.40 (0.94 to 1.86)	18.0 (15.2 to 20.7)
Non-smokers	22	1.38 (0.72 to 2.04)	17.6 (14.1 to 21.1)
Smokers	11	1.45 (0.89 to 2.01)	18.8 (13.4 to 24.2)
Engine room personnel			
No oil on skin	15	2.84 (1.52 to 4.14)	18.7 (14.6 to 22.7)
Non-smokers	6	1.18 (0.23 to 2.13)	19.5 (9.9 to 29.1)
Smokers	9	3.94 (2.09 to 5.79)	18.2 (13.3 to 23.0)
Oil on skin	19	5.25 (3.10 to 7.39)	23.2 (18.6 to 27.8)
Non-smokers	13	5.44 (2.31 to 8.58)	21.8 (16.5 to 27.0)
Smokers	6	4.82 (2.16 to 7.48)	26.3 (14.0 to 38.6)

Table 3 Regression analysis of determinants for urine concentration of ln 8OHdG (nmol/l), adjusted to density 1.022

	Unstandardised coefficient	SE	Standardised coefficient	p
Ln 1-hydroxypyrene in urine*	0.12	0.052	0.31	0.03
Work in engine room	−0.08	0.14	−0.09	0.57
Oil on skin	0.16	0.15	0.17	0.29
Smoking category	−0.003	0.036	−0.01	0.92
Age	−0.007	0.005	−0.17	0.23

*The natural logarithm of the concentration of 1-hydroxypyrene in urine (nmol/l), adjusted to density 1.022.

physical exercise is reported to be beneficial, while extreme physical activities could increase the levels.[34] We did not observe any major difference in workload between the exposed group and the controls. The difference in mean creatinine concentrations between the exposed group and the control group could possibly, at least partly, be explained by desiccation due to the higher temperature in the engine room.

The excretion of 1OHP may be increased by the intake of food rich in PAH.[21 35] Dietary factors can probably also influence the excretion of 8OHdG. However, the engine room personnel and the control group had the same food on the ships. It is therefore unlikely that the diet influenced the results to any great extent.

Tobacco smoke may influence the results of both 1OHP and 8OHdG.[25 26 35] Other studies have, however, failed to show an effect of smoking on the urinary excretion of 8OHdG.[22 30] It is unlikely that any effect of smoking influences our results as the exposed group and the controls had similar smoking habits. The influence of tobacco smoke was also accounted for in the multiple regression analyses.

Age could influence the excretion of 8OHdG,[36] and the association with exposure was increased when age was included in the model in the multiple regression analysis.

It is likely that reactive oxygen species are formed during the metabolism of PAH, such as benzo[a]pyrene.[15] Urinary concentrations of 1OHP and 8OHdG have been analysed in a study of potroom workers[30] and in a study of roofers.[37] The urinary levels of 1OHP among the exposed worker in both of these studies were considerably higher than in our study. In the study of potroom workers, no significant correlation was found between any of the exposure measures and 8OHdG in urine. In the study of roofers a small, but statistically significant increase in 8OHdG was evident in end-of-week urine samples compared with start-of-week urine samples in roofers exposed to coal tar. The increase in urinary 8OHdG was accompanied by a decrease in leucocyte 8OHdG/dG, suggesting that PAH from coal tar exposure induces increased antioxidant capacity or repair mechanisms. If this is the case, it could be a possible explanation for the finding that the two subjects with the highest urinary levels of 1OHP in our study had comparatively low urinary excretion of 8OHdG (fig 1). Exclusion of these two subjects from the analysis only marginally affected the results.

The urinary concentrations of 1OHP (adjusted for creatinine) in this study of engine room personnel were lower (0.11, 0.17, and 0.37 µmol/mol creatinine for exposure groups 0, I, and II respectively) than the levels reported from coke oven workers[10 11 38–40] and aluminium workers,[41] but similar to values for car repair workers[42] and boilermakers.[43] The uptake is not only dependent on the PAH content, but also on the matrix.[44]

The exposure levels in our study were considerably lower than in most previous studies reporting genotoxic effects such as DNA single strand breakage, DNA adducts, and sister chromatid exchanges in lymphocytes.[12] The levels of 8OHdG in urine were higher in this study than in a previous study of car repair workers, refinery workers, and gasoline pump repairmen occupationally exposed to gasoline.[22]

Several compounds containing PAH and/or nitroarenes are classified as carcinogens.[45–48] There are indications that 8OHdG may have a role in mutagenesis and carcinogenesis.[17 19] Dermal exposure to PAH may contribute to an increased risk of cancer of the skin and possibly urinary bladder. An increased risk of lung cancer cannot be ruled out since PAH-DNA adducts have been found in white blood cells and the lungs after dermal application of benzo[a]pyrene, tar, and bitumen products on the skin of mice.[49 50]

Since this study is the first to indicate an association between exposure to oils in engine rooms and an increased excretion of a biomarker of oxidative stress, the results have to be corroborated in further studies before any firm conclusions can be drawn.

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REFERENCES

1 **Tola S**, Tenho M, Korkala M-L, *et al*. Cancer of the urinary bladder in Finland. *Int Arch Occup Environ Health* 1980;**46**:43–51.
2 **Malker HSR**, McLaughlin JK, Silverman DT, *et al*. Occupational risks for bladder cancer among men in Sweden. *Cancer Res* 1987;**47**:6763–6.
3 **Silverman DT**, Levin LI, Hoover RN, *et al*. Occupational risks of bladder cancer in the United States. *J Natl Cancer Inst* 1989;**81**:1472–80.
4 **Dolin PJ**, Cook-Mozaffari P. Occupation and bladder cancer. *Br J Cancer* 1992;**66**:568–78.
5 **Brandt LPA**, Kirk NU, Jensen OC, *et al*. Mortality among Danish merchant seamen from 1970 to 1985. *Am J Ind Med* 1994;**25**:867–76.
6 **Rafnsson V**, Gunnarsdóttir H. Cancer incidence among seamen in Iceland. *Am J Ind Med* 1995;**27**:187–93.
7 **Mastrangelo G**, Fadda E, Marzia V. Polycyclic aromatic hydrocarbons and cancer in man. *Environ Health Perspect* 1996;**104**:1166–70.
8 **Becher G**, Bjørseth A. Determination of occupational exposure to PAH by analysis of body fluids. In: Bjørseth A, Ramdahl T, eds. *Handbook of polycyclic aromatic hydrocarbons*, Vol. 2. New York: Marcel Dekker, 1985:237–52.
9 **Jongeneelen FJ**, Bos RP, Anzion RBM, *et al*. Biological monitoring of polycyclic aromatic hydrocarbons; metabolites in urine. *Scand J Work Environ Health* 1986;**12**:137–43.
10 **Tolos WP**, Shaw PB, Lowry LK, *et al*. 1-Pyrenol, a biomarker for occupational exposure to polycyclic aromatic hydrocarbons. *Appl Occup Environ Hyg* 1990;**5**:303–9.
11 **Buchet JP**, Gennart JP, Mercado-Calderon F, *et al*. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. *Br J Ind Med* 1992;**49**:761–8.
12 **Jongeneelen FJ**. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann Occup Hyg* 2001;**45**:3–13.

13 **Sbrana I**, Puliti A, Seidel A, *et al.* Induction of chromosomal aberrations and spindle disturbances in Chinese hamster epithelial liver cells in culture by pyrene and benzo[a]pyrene quinones. *Mutagenesis* 1995;**10**:505–12.
14 **Flowers-Geary L**, Bleczinski W, Harvey RG, *et al.* Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon *o*-quinones produced by dihydrodiol dehydrogenase. *Chem Biol Interact* 1996;**99**:55–72.
15 **Yang Y**. The in vivo metabolism of benzo[a]pyrene studied by chromatography in combination with mass spectrometry [dissertation]. Stockholm: Department of Medical Nutrition, Karolinska Institute, 1997.
16 **Kasai H**, Crain PF, Kuchino Y, *et al.* Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 1986;**7**:1849–51.
17 **Floyd RA**. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis* 1990;**11**:1447–50.
18 **Shigenaga MK**, Gimeno CJ, Ames BN. Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proc Natl Acad Sci* 1989;**86**:9697–701.
19 **Ames BN**. Endogenous DNA damage as related to cancer and ageing. *Mut Res* 1989;**214**:41–6.
20 **Moen BE**, Nilsson R, Nordlinder R, *et al.* Assessment of exposure to polycyclic aromatic hydrocarbons in engine rooms by measurement of urinary 1-hyroxypyrene. *Occup Environ Med* 1996;**53**:692–6.
21 **Buckley TJ**, Lioy PJ. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *Br J Ind Med* 1992;**49**:113–24.
22 **Nilsson RI**, Nordlinder RG, Tagesson C, *et al.* Genotoxic effects in workers exposed to low levels of benzene from gasoline. *Am J Ind Med* 1996;**30**:317–24.
23 **Jongeneelen FJ**, Anzion RBM, Henderson PT. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatogr* 1987;**413**:227–32.
24 **Tagesson C**, Källberg M, Leanderson P. Determination of urinary 8-hydroxydeoxyguanosine by coupled-column high-performance liquid chromatography with electrochemical detection: a noninvasive assay for in vivo oxidative DNA damage in humans. *Toxicology Methods* 1992;**1**:242–51.
25 **Tagesson C**, Källberg M, Wingren G. Urinary malondialdehyde and 8-hydroxydeoxyguanosine as potential markers of oxidative stress in industrial art glass workers. *Int Arch Occup Environ Health* 1996;**69**:5–13.
26 **Loft S**, Vistisen K, Ewertz M, *et al.* Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* 1992;**13**:2241–7.
27 **Alessio L**, Berlin A, Dell'Orto A, *et al.* Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *Int Arch Occup Environ Health* 1985;**55**:99–106.
28 **Trevisan A**. Concentration adjustment of spot samples in analysis of urinary xenobiotic metabolites. *Am J Ind Med* 1990;**17**:637–42.
29 **Boeniger MF**, Lowry LK, Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am Ind Hyg Assoc J* 1993;**54**:615–27.
30 **Carstensen U**, Hou S-M, Alexandrie A-H, *et al.* Influence of genetic polymorphisms of biotransformation enzymes on gene mutations, strand breaks of deoxyribonucleic acid, and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine in potroom workers exposed to polyaromatic hydrocarbons. *Scand J Work Environ Health* 1999;**25**:351–60.
31 **Pilger A**, Germadnik D, Riedel K, *et al.* Longitudinal study of urinary 8-hydroxy-2′-deoxyguanosine excretion in healthy adults. *Free Radic Res* 2001;**35**:273–80.
32 **Kasai H**. Chemistry-based studies on oxidative DNA damage: formation, repair and mutagenesis. *Free Radic Biol Med* 2002;**33**:450–6.
33 **Murata M**, Yoshiki Y, Tada M, *et al.* Oxidative DNA damage by a common metabolite of carcinogenic nitrofluorene and n-acetylaminofluorene. *Int J Cancer* 2002;**102**:311–17.
34 **Sato Y**, Nanri H, Ohta M, *et al.* Increase of human MTH1 and decrease of 8-hydroxydeoxyguanosine in leukocyte DNA by acute and chronic exercise in healthy male subjects. *Biochem Biophys Res Commun* 2003;**305**:333–8.
35 **Van Rooij JGM**, Bodelier-Bade MM, Hopmans PMJ, *et al.* Reduction of urinary 1-hydroxypyrene excretion in coke-oven workers exposed to polycyclic aromatic hydrocarbons due to improved hygienic skin protective measures. *Ann Occup Hyg* 1994;**38**:47–56.
36 **Kasai H**, Iwamot-Tanaka N, Miyamoto T, *et al.* Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index and smoking. *Jpn J Cancer Res* 2001;**92**:9–15.
37 **Toraason M**, Hayden C, Marlow D, *et al.* DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. *Int Arch Occup Environ Health* 2001;**74**:396–404.
38 **Jongeneelen FJ**, Anzion RBM, Scheepers PTJ, *et al.* 1-hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments. *Ann Occup Hyg* 1988;**32**:35–43.
39 **Øvrebø S**, Fjeldstad PE, Grzybowska E, *et al.* Biological monitoring of polycyclic aromatic hydrocarbon exposure in a highly polluted area of Poland. *Environ Health Perspect* 1995;**103**:838–43.
40 **Øvrebø S**, Haugen A, Farmer PB, *et al.* Evaluation of biomarkers in plasma, blood, and urine samples from coke oven workers: significance of exposure to polycyclic aromatic hydrocarbons. *Occup Environ Med* 1995;**52**:750–6.
41 **Øvrebø S**, Haugen A, Hemminki K, *et al.* Studies of biomarkers in aluminium workers occupationally exposed to polycyclic aromatic hydrocarbons. *Cancer Detect Prev* 1995;**19**:258–67.
42 **Granella M**, Clonfero E. Urinary excretion of 1-pyrenol in automotive repair workers. *Int Arch Occup Environ Health* 1993;**65**:241–5.
43 **Mukherjee S**, Rodrigues E, Weker R, *et al.* 1-hydroxypyrene as a biomarker of occupational exposure to polycyclic aromatic hydrocarbons (PAH) in boilermakers. *J Occup Environ Med* 2002;**44**:1119–25.
44 **Sartorelli P**, Cenni A, Matteucci G, *et al.* Dermal exposure assessment of polycyclic aromatic hydrocarbons: in vitro percutaneous penetration from lubricating oil. *Int Arch Occup Environ Health* 1999;**72**:528–32.
45 **International Agency for Research on Cancer**. *IARC monographs on the evaluation of carcinogenic risks to humans: risks to humans: polynuclear aromatic compounds. Part 1: chemical environmental and experimental data.* Lyon: IARC, 1984;**33**:95–447.
46 **International Agency for Research on Cancer**. *IARC monographs on the evaluation of carcinogenic risks to humans: polynuclear aromatic compounds. Part 2. Carbon black, mineral oils and some nitroarenes.* Lyon: IARC, 1984;**33**:93–7.
47 **International Agency for Research on Cancer**. *IARC monographs on the evaluation of carcinogenic risks to humans: occupational exposures in petroleum refining; crude oil and major petroleum fuels.* Lyon: IARC, 1989;**45**:239–49.
48 **International Agency for Research on Cancer**. *IARC monographs on the evaluation of carcinogenic risks to humans: diesel and gasoline, engine exhaust, and some nitroarenes.* Lyon: IARC, 1989;**46**:47–58.
49 **Godschalk RWL**, Moonen EJC, Schilderman PAEL, *et al. Carcinogenesis* 2000;**1**:87–92.
50 **Schoket B**, Hewer A, Grover PL, *et al.* Formation of DNA adducts in human skin maintained in short-term organ culture and treated with coal-tar, creosote or bitumen. *Int J Cancer* 1988;**42**:622–6.